This document contains TSCA Confidential Business Information

MCAN SOP: [redacted]

Lab gloves, safety goggles and lab coat required unless stated otherwise.

Sample Collection: This protocol applies to fermentation broth samples from seed culture to final fermentor tanks

1. Collect [redacted] sample in a [redacted] Corning tube as aseptically as possible.
2. In a biohood, transfer [redacted] f sample to a [redacted] Eppendorf tube for cell count purposes.
3. Remainder sample to be used for plating.

Cell Count (lab gloves optional)

1. From the [redacted] sample, load [redacted] onto each side of a [redacted] Capillary action should pull the cells towards the center chamber, evenly covering the area.

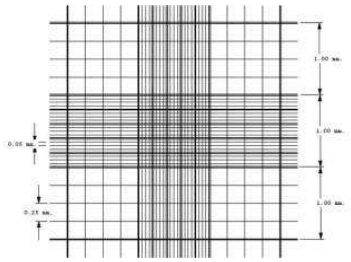
2. If cells are too confluent, make a dilution from the [redacted] sample. Use water and note the dilution factor. Cell counts beyond [redacted] will require dilutions. Seed cultures at an [redacted] equal to [redacted] will have cell counts in the [redacted] range, with fermentors achieving [redacted] equal to 50-60 and cell counts in excess of [redacted] data from [redacted].

3. Place [redacted] onto microscope stage. Use the 10X objective to focus.
   a. Cells from late stage fermentation [redacted] Focus and count through multiple planes to achieve a more accurate cell count.
   b. [redacted] count as 1 cell.

4. Count a minimum of 2 squares (4x4 grid, 1mm x 1mm, see below) and average the numbers.
5. Calculate Total cells/mL:

   Total cells/mL = (cell count)(dilution factor)(10,000)

6. Discard the sample used for cell counting.



[redacted]

Plating (in biohood)

1. Open the [redacted] and allow plates and lids to dry until any moisture is evaporated.
2. Label bottom of the plates with Strain, Date, Plate Type and any other identifying info.
3. Based on total cells/mL, dilute the remainder sample. Use a 6 well plate and [redacted]
   Dilution should aim for theoretical [redacted] colonies per plate with a [redacted] plating volume:

4. Add [redacted] of the diluted sample onto a plate.
5. Add 4-6 sterile plating beads. Cover plate w/lid and quickly shake side to side to spread the sample.
6. Plate all samples in duplicate.
7. Discard plating beads. Leave plates open to dry for 5 minutes.

Incubation

1. Close plates and remove from biohood. Wrap plates with parafilm.
2. Incubate plates upside down at [redacted]
3. Colonies (if any) should be visible after [redacted] days.

Plate Count & Viability

1. Once colonies are visible, remove plates from incubator. Image plates (optional).

2. Use an eCount pen to count number of colonies on each plate (CFU).
3. If replicates were plated, average the CFU/mL of all plates. Exclude any obvious plating errors.
   Determine CFU/mL:

   [redacted]
4. Calculate % viability:
   [redacted]
5. >100% viability may be possible due to inaccurate cell counts, especially [redacted] These types of results are considered 100% viable. Dilution or plating errors may also contribute to inaccurate viability assessment so careful pipetting is required.